

Claim Amendments and Status

1-73. (Cancelled).

74. (New) A method for identifying a polynucleotide sequence that encodes a peptide which binds to a preselected receptor molecule, comprising:

transforming host cells by electroporation with at least 10^8 different filamentous bacteriophage expression vectors, wherein each of said different vectors comprises a polynucleotide sequence that encodes a fusion protein comprising a peptide fused to a coat protein of a filamentous bacteriophage so that the N-terminal amino acid of said fusion protein is the N-terminal amino acid of said peptide, and wherein said different vectors are constructed by ligating each polynucleotide of a mixture of at least 10^8 different polynucleotides to a bacteriophage cloning vector that encodes the coat protein so as to form a coding sequence for said fusion protein, wherein each of said different polynucleotides comprises a series of codons encoding a random collection of amino acids and encodes a different peptide;

cultivating the transformed cell under conditions suitable for expression and assembly of bacteriophage particles thereby displaying said peptides on the surface of said particles;

selecting bacteriophage particles displaying the peptide by combining said particles with the preselected receptor molecule and separating particles bound to said receptor molecule from unbound particles; and

sequencing said polynucleotide sequence encoding said peptide within said selected bacteriophage particles.

75. (New) A method for identifying a polynucleotide sequence that encodes a peptide which binds to a preselected receptor molecule, comprising:

transforming host cells by electroporation with at least 10^8 different filamentous bacteriophage expression vectors, wherein each of said different vectors comprises a polynucleotide sequence that encodes a fusion protein comprising a peptide fused to a coat protein of a filamentous bacteriophage, and wherein said different vectors differ from each other with respect to the peptide of said fusion protein encoded by said vector; cultivating the transformed cell under conditions suitable for expression and assembly of bacteriophage particles thereby displaying said peptides on the surface of said particles; selecting bacteriophage particles displaying the peptide by combining said particles with the preselected receptor molecule and separating particles bound to said receptor molecule from unbound particles; and

sequencing said polynucleotide sequence encoding said peptide within said selected bacteriophage particles;

wherein each of said 10^8 different polynucleotides is comprised of first, second, and third single-stranded oligonucleotides that are annealed to one another prior to insertion into said cloning vector; and

wherein said first single stranded oligonucleotide is .sup.5' CTCTCACTCC (NNK).sub.x ACTGTTGAAGA GTTG T; and said second and third single stranded oligonucleotides are .sup.5' GGAGTGAGAGTAGA and .sup.5' CTTTCAACA GT, respectively.

76. (New) A method for identifying a polynucleotide sequence that encodes a peptide which binds to a preselected receptor molecule, comprising:

transforming host cells by electroporation with at least 10^6 different filamentous bacteriophage expression vectors, wherein each of said different vectors comprises a polynucleotide sequence that encodes a fusion protein comprising a peptide fused to a coat protein of a filamentous bacteriophage, and wherein said different vectors differ from each other with respect to the peptide of said fusion protein encoded by said vector; cultivating the transformed cell under conditions suitable for expression and assembly of bacteriophage particles thereby displaying said peptides on the surface of said particles;

selecting bacteriophage particles displaying the peptide by combining said particles with the preselected receptor molecule and separating particles bound to immobilized preselected receptor molecule from unbound particles;

repeating the selection step at least once, wherein the selected bacteriophage particles are propagated between said selection steps and wherein said receptor is immobilized at reduced densities in subsequent repetitions of the selecting step; and

sequencing said polynucleotide sequence encoding said peptide within said selected bacteriophage particles.